Food Anal. Methods (2012) 5:1167–1176
DOI 10.1007/s12161-011-9349-3

Multivitamin Analysis of Fruits, Fruit–Vegetable Juices, and Diet Supplements

Joanna Płonka · Agata Toczek · Violetta Tomczyk

Received: 30 July 2011 / Accepted: 12 December 2011 / Published online: 13 January 2012

Abstract Vitamins are organic compounds that are required for various biological functions. In general, vitamins are not synthesized in the human body, but lack or deficiency of them may lead to certain diseases. Determinations of 11 vitamins in various products were performed, which included ascorbic acid (C), seven vitamins of the B group (thiamine B_1, riboflavin B_2, nicotinamide B_3, pantothenic acid B_5, pyridoxine B_6, folic acid B_9, and cyanocobalamin B_{12}), as well as three fat-soluble vitamins (retinol A, cholecalciferol D_3, and α-tocopherol E). A column with RP18 stationary phase and a diode array detector with properly selected analytical wavelengths for each compound were used. A gradient of trifluoroacetic acid in water with methanol was used as the mobile phase. Limits of quantification in the range of 0.70–2.90 μg/mL for water-soluble vitamins and 1.85–15.84 μg/mL for fat-soluble vitamins were obtained. Those values are sufficient for determinations of the aforementioned compounds in foodstuff. The developed procedure of sample preparation together with chromatographic system can be used for food quality monitoring in the food industry.

Keywords Water-soluble vitamins · Fat-soluble vitamins · Fruits · Juices · High-performance liquid chromatography · SPE

Introduction

Vitamins are a vital group of food components that must be provided to the human body in sufficient amounts. They support metabolism processes and improve the efficiency of proteins and enzymes. Vitamins are provided in final form or as provitamins that are subsequently transformed into vitamins. The main sources of vitamins are fruits, vegetables, meats, and fish. However, when acquisition from food is insufficient, the vitamins can be acquired from pharmaceutical preparations. Currently, the increased interest in a balanced and wholesome diet has caused increased demand for multivitamin diet supplements.

Vitamins are divided into two groups based on their solubility. The fat-soluble vitamins A, D_3, E, and K are non-polar, hydrophobic compounds that are isoprene derivatives. They are absorbed by the human organism also with fat and can be stored in liver, kidneys, and fat tissue. Fat-soluble vitamins perform essential roles in the regulation of height, calcium and phosphorus absorption, and the development and functionality of bones. They also act as anticoagulants. The water-soluble vitamin group contains vitamin C (ascorbic acid), B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinamide), B_5 (pantothenic acid), B_6 (pyridoxine), B_9 (folic acid), and B_{12} (cyanocobalamin). They have different chemical structures because they represent acid, pyrimidine, and imidazole derivatives as well as acid amides. They are responsible for the proper functioning of the nervous and respiratory systems, synthesis of nucleic and fatty acids, and creation of red blood cells (National Academy of Sciences 1989; Sikorski 2007). Lack or deficiency of vitamins in consumed food can lead to deficiency states and diseases.

A widely used analytical method for the determination of vitamins in food and diet supplements is chromatography, especially high-performance liquid chromatography (HPLC). HPLC is described by high resolution and selectivity, short time of analysis, and ability to couple with other techniques. Literature publications describe numerous methods for vitamin determination; however, particular

J. Płonka (✉) · A. Toczek · V. Tomczyk
Department of Analytical Chemistry,
Silesian University of Technology,
7 M. Strzody Str.,
44-100 Gliwice, Poland
e-mail: joanna.plonka@polsl.pl

procedures allow only determining one, two, or several vitamins at the same time, but only from either the fat- or water-soluble group. Table 1 shows parameters of chromatographic procedures for the determination of fat- and water-soluble vitamins. Literature data allow the selection of a chromatographic procedure that enables the determination of vitamins in food samples with adequate concentration levels with the use of both gradient and isocratic systems. The disadvantage of the described procedures is the small number of analytes that can be determined at the same time. Most of the methods allow the determination of several vitamins from one group at once. Most often, it is vitamin A together with E or three to four vitamins of the B group. Only in single particular cases does a chromatographic procedure allow for the simultaneous separation of several compounds of both fat- and water-soluble groups without requirement of stationary or mobile phase changes.

Another issue is proper food sample preparation, which would allow the simultaneous extraction of all analytes from a matrix. Solid–liquid and solid phase extraction are the most often used extraction techniques. Most of the procedures described in the literature concern single vitamins of water-soluble vitamins (Hussein et al. 2000; Kurilich et al. 1999; Wang et al. 1996; Franke et al. 2004; Frenich et al. 2005; Gil et al. 2006; Mahattanatawee et al. 2006; Wall 2006; Singh et al. 2007; Lopez-Berenguer et al. 2009) and fat-soluble vitamins (Kurilich et al. 1999; Mahattanatawee et al. 2006; Singh et al. 2007; Ching and Mohamed 2001). Only two papers (Ndaw et al. 2000; Lebiedzińska and Szefer 2006) describe procedures that allow the simultaneous extraction of three to four vitamins from one group. Other studies (Moreno and Salvadó 2000; Munzuroglu et al. 2003) show that it is possible to extract vitamins from both groups; however, this requires a few additional changes in the procedures, like adding a secondary solvent.

Our study presents results of the development of an analytical procedure for the simultaneous extraction as well as simultaneous chromatographic determination of fat- and water-soluble vitamins. After an examination of standard samples, application to real samples was performed. The developed method allows reduction in time for the analysis of fruits, vegetables, and juices, as well as multivitamin diet supplements.

Materials and Methods

Reagents

Standard solutions (10 mg/mL) of retinol (A), cholecalciferol (D_3), α-tocopherol (E), thiamine (B_1), riboflavin (B_2), nicotinamide (B_3), pantothenic acid (B_5), pyridoxine (B_6), folic acid (B_9), cyanocobalamin (B_{12}), and ascorbic acid (C) (all from Sigma, St. Louis, MO, USA) were prepared in methanol. Methanol, trifluoroacetic acid (TFA), and water of HPLC grade used for the mobile phase were purchased from Merck (Darmstadt, Germany). Acetic acid 99%, zinc acetate, and potassium ferricyanide (all from POCH, Gliwice, Poland) of analytical grade were used. Standard solutions were prepared directly before use.

High-Performance Liquid Chromatography Conditions

HPLC analysis was performed using a Merck-Hitachi chromatograph equipped with an L6200A pump, L-7360 thermostat, and L4500A diode array detector. Chromatographic separation was carried out on a TSKGel ODS-100V, 150×4.6 mm (5 μm) column (Tosoh Bioscience). Examination was performed at a temperature of 30 °C. A gradient elution with 0.01% TFA in water (A) and methanol (B) was applied. The gradient elution parameters are shown in Table 2.

Detection of vitamins was performed using a diode array detector (DAD) with analytical wavelengths typical for each analyte: retinol, 320 nm; cholecalciferol, 275 nm; thiamine, 253 nm; riboflavin, α-tocopherol, and cyanocobalamin, 290 nm; nicotinamide, 258 nm; pantothenic acid, 218 nm; pyridoxine, 289 nm; folic acid, 360 nm; and ascorbic acid, 262 nm.

Calibration Curves

The calibration curves for the examined compounds were prepared in a matrix of methanolic food extract. Matrix of food samples was purified by solid phase extraction with the same parameters as the extraction of analytes (see "Sample Preparation"). The effluent formed during sample passing through solid phase was collected. Then, appropriate amounts of standard solutions were added.

Curves were determined in a concentration range appropriate for the expected content of analytes in real food samples. The number of experimental points taken for regression was $n=6$. Every analyte was injected three times. The volume of each injection was 20 μL. Values of standard deviation of slope and intercept for calibration curves were calculated.

Carrez I and Carrez II Solution Preparation

Carrez I solution was prepared by dissolving 10.6 g of potassium ferricyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) in 100 mL of distilled water. Carrez II solution was prepared by dissolving 21.95 g of zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$) and 30 mL of 99% acetic acid in 100 mL of distilled water.

Table 1 Procedures for the selected chromatographic determination methods of vitamins in foodstuff

No.	Vitamins	Mobile phase	Stationary phase	Detector	LOD (ng/mL)	Measurement range (μg/mL)	Sample type	Reference
1	A D	A: 0.010% TFA (pH 3.9) B: MeOH	C18. 150×4.6 mm (3 μm)	DAD MS	12.30–12.49 41.77–129.4	–	Vitamin preparations	Klejdus et al. (2004)
	E	Gradient elution 35 min			34.78–127.30			
	K				83.10–197.40			
	B_1				12.18			
	B_2				188.00			
	B_3				16.41–35.40			
	B_6				21.17–23.08			
	B_9				30.48			
	B_{12}				97.47			
	C				25.55			
2	B_1 B_2	A: 0.4 M $LiClO_4$ (pH 2.4) B: CH_3CN	C18, 75×2 mm (5 μm)	UV	–	–	Vitamin preparations	Kozhanova et al. (2002)
	B_3 B_5	Gradient elution 25 min						
	B_6							
	B_7							
	B_9							
	B_{12}							
	A D_2	CH_3CN/H_2O (95:5, *v*/*v*)						
	D_3							
	E							
	K_3							
3	B_1 B_2	A: 0.05 M CH_3COONH_4 B: MeOH	C18. 150×3.9 mm (4 μm)	UV–Vis	3.18 1.84	4.93–39.44 2.18–17.43	Vitamin preparations	Moreno and Salvadó (2000)
	B_3	Gradient elution 17 min			9.92	17.53–140.24		
	B_6				1.37	1.78–14.20		
	B_{12}				0.04 (LOQ)	0.04–0.12		
	A	CH_3CN/MeOH (95:5. *v*/*v*)			5.00	4.94–39.49		
	E				3.09	10.15–81.20		
	D3				0.05	0.16–1.30		
4	B_1 B_2	A: 5 mM HFBA B: MeOH	C18. 250×4.6 mm (5 μm)	MS	3 6	0.01–50 0.01–50	Vitamin preparations	Chen et al. (2006)
	B_3	Gradient elution 30 min			8–10	0.02–50		
	B_5				8	0.02–50		
	B_6				1–3	0.01–50		
	B_7				3	0.02–50		
	B_9				9	0.01–50		
	C				12	0.02–50		
5	B_1 B_2	A: 0.025% TFA (pH 2.6) B: CH_3CN	C18, 250×4.6 mm (5 μm)	UV	–	1.25–50 0.62–25	Infant foodstuff	Heudi et al. (2005)
	B_3	Gradient elution 17 min				1.25–50		
	B_5					2.5–500		
	B_6					0.125–50		
	B_8					0.5–50		
	B_9					0.6–25		
	B_{12}					0.25–10		
	C					5–200		
6	B_1	A: 16 mM SDS+0.02 M phosphate buffer (pH 3.6)+3.5% BuOH	C18. 150×4.6 mm (7 μm)	UV	17,000	125–400	Vitamin preparations	Ghorbani et al. (2004)
	B_2	B: 16 mM SDS+0.02 M phosphate buffer (pH 3.6)+10% BuOH	C18. 250×4.6 mm (10 μm)		4,000	11–68		
	B_3	Gradient elution 75 min			20,000	95–750		
	B_6				5,000	12–140		
	B_9				7,100	50–175		

Table 1 (continued)

No.	Vitamins	Mobile phase	Stationary phase	Detector	LOD (ng/mL)	Measurement range (μg/mL)	Sample type	Reference
	B_{12}				120	0.2–1.3		
	C				50,000	100–1500		
7	B_1	A: 0.1% HCOOH	C18. 220×2 mm	DAD	20	0.1–2.0	Vitamin preparations	Chen and Wolf (2007)
	B_2	B: 0.1% HCOOH in CH_3CN	(5 μm)	MS	20	0.1–10		
	B_3	Gradient elution 25 min			100	1.0–100.0		
	B_5				20	0.5–50		
	B_6				20	0.1–10.0		
	B_7				20	0.02–0.2		
	B_9				50	0.2–1.0		
8	B_1	A: 0.0125 M $CH_3(CH_2)_5SO_3Na$ in 0.1% H_3PO_4 (pH 2.4–2.5)	C18. 250×4.6 mm (5 μm)	DAD	100	18.4–50.4	Medicated syrup	Vidović et al. (2008)
	B_2	B: CH_3CN			200	7.7–21.6		
	B_3	Gradient elution 60 min			200	119.8–359.0		
	B_5				700	50.3–165.9		
	B_6				100	6.0–17.9		
	C				400	964.0–3004.0		
9	B_1	A: MeOH	Amid C16. 150×4.6 mm	MS	1	0.005–10	Pasta	Leporatia et al. (2005)
	B_2	B: 20 mM $HCOONH_4$ (pH 3.75)	(5 μm)		3–5	0.005–12.5		
	B_3	Gradient elution 12 min			0.5	0.01–25		
	B_5				5	0.01–50		
	B_6				1–3	0.005–25		
	B_9				5	0.01–50		
10	B_1	A: Phosphate buffer (pH 6)	Amid C16	DAD	10	0.05–1.00	Baby food	Viñas et al. (2003)
	B_2	B: CH_3CN	(5 μm)		3	0.02–1.0		
	B_3	Gradient elution 45 min			19–38	0.07–1.5		
	B_6				19–20	0.07–1.5		
	B_9				5	0.02–1.0		
	B_{12}				10	0.05–1.0		
11	B_1	A: 0.1 M SDS	C18. 120×4.6 mm	UV	20	0.5–25	Vitamin preparations	Monferrer-Pons et al. (2003)
	B_2	B: 4% PnOH in 0.1 M phosphate buffer (pH 3)	(5 μm)		3	0.5–25		
	B_3	Gradient elution 22 min			10	0.5–25		
	B_6				5–12	0.5–25		
12	B_1	A: 0.1% HCOOH	Hydro-RP 250×2 mm	MS	–	–	Vitamin preparations	Chen et al. (2007)
	B_3	B: 0.1% HCOOH in CH_3CN	(4 μm)					
	B_5	Gradient elution 25 min						
	B_6							
13	B_1	Phosphate buffer/MeOH (90:10, *v/v*) adjusted to pH 3.55 with 0.018 M TMA+85% H_3PO_4	C18. 250×4.6 mm (5 μm)	DAD	9.2	22–8200	Juices, sea fruit, vitamin preparations	Lebiedzińska et al. (2007); Marszałł et al. (2005)
	B_6			ED	0.19–0.29	9.5–2800		
	B_{12}				0.0021	2–25		
14	B_1	A: 0.5% TEA+15% MeOH+2.4% CH_3COOH+5 mM OSA	C8, 250×4.6 mm (5 μm)	DAD	50	–	Meat	Riccio et al. (2006)
	B_6	B: CH_3CN			50			
	B_{12}	Gradient elution 17 min			50			
15	A	A: MeOH/H_2O (90:10, *v/v*)	C18. 250×2 mm	MS	0.0025	0.097–1.783	Human milk	Kamao et al. (2007)
	D_2	B: CH_3CN	(5 μm)		0.00005	0.010–1.116		
	D_3	Gradient elution 30 min			0.00005	0–1.300		
	E	For D_3—A :CH_3CN/H_2O (30:70, *v/v*)			0.005	0.387–35.664		
	K_1	B: CH_3CN			0.0005	0.953–12.382		
	K_2	Gradient elution 35 min			0.0005–0.004	0.074–15.861		
16	A	A: MeOH/H_2O (99:1, *v/v*)	C18, 150×0,3 mm	UV	0.02	–	Milk	Gomis et al. (2000)
	D_2	B: MeOH/THF (70:30, *v/v*)	(3 μm)		2			
	D_3	Gradient elution 16 min			0,2			
	E				2			
	K_1				0.4			

Table 1 (continued)

No.	Vitamins	Mobile phase	Stationary phase	Detector	LOD (ng/mL)	Measurement range (μg/mL)	Sample type	Reference
17	A D_3 E K_1	3% SDS+15% BuOH adjusted to pH 7 with 0.02 M phosphate buffer	C18. 250×4.6 mm (5 μm)	DAD	810 910 1120 830	1.0–60 1.0–40 1.0–60 1.0–60	Food, vitamin preparations	Kienen et al. (2008)
18	A D_2 D_3 E	MeOH/H_2O (99:1, *v*/*v*)	C18, 120×2 mm (5 μm)	UV–Vis	–	–	Vitamin preparations	Kozlov et al. (2003)
19	A C E	MeOH	C18, 125×4 mm (5 μm)	UV–Vis	15–36 65 53	1.6–7.7 5.0–100.7 0.04–0.19	Vitamin preparations	Paulo et al. (1999)
20	A D_3 E	Heksan+dioksan+i-PrOH (96.7:3:0.3, *v*/*v*/*v*)	C18 250×4.6 mm	MS	0.0014 0.000008 0.00025	0.15–12 0.005–0.4 0.25–20	Infant nutrition	Heudi et al. (2004)
21	A E K_1	MeOH/H_2O (50:50, *v*/*v*)	C18. Monolith 270×10 mm	UV–Vis	3.9 173 16.4	0.2–2 1–10 0.2–2	Cereals	Xu and Jia (2009)
22	D_3 E	CH_3CN with Chl+H_2TPP	C18. 300×3.9 mm (5 μm)	UV	3.5–2300 120	–	Vitamin preparations	Lazareva et al. (2002)
23	A E	MeOH/H_2O (96:4, *v*/*v*)	C18, 250×4 mm (5 μm) RP-60 select B 250×4 mm (5 μm)	UV FD	–	–	Meat, milk, powdered milk	Berg et al. (2000)
24	A E	A: CH_3CN/0.04% TFA (1:1, *v*/*v*) B: CH_3CN/MeOH (3:7, *v*/*v*) Gadient elution 15 min	C18, 120×2 mm (5 μm)	UV	–	–	Vitamin preparations	Kozlov et al. (2004)
25	A E	A: MeOH/H_2O (96:4, *v*/*v*) B: TBME/MeOH/H_2O (90:6:4, *v*/*v*/*v*) Gradient elution 30 min	C30. 250×4.6 mm (5 μm)	DAD MS	100–1,100 (LOQ) 100–10,000 (LOQ)	10–400 10–400	Vitamin preparations	Breithaupt and Kraut (2006)
26	A E	MeOH/H_2O (96:4, *v*/*v*)	C18, 250×4 mm (5 μm)	UV	–	–		Turner and Mathiasson (2000)
27	A E	MeOH	C18, 250×4.6 mm (5 μm)	DAD	0.33 3.2–32.9	5–100 5–100	Milk	Rodas Mendoza et al. (2003)
28	A E	0.5% $CH_3COOC_2H_5$/hexane (1:1, *v*/*v*)	50×2.1 mm (3 μm)	DAD	0.2–0.3 4.9–8.8	0.2–5 1–100	Baby food	Chávez-Servín et al. (2006)
29	A E	MeOH/H_2O (96:4, *v*/*v*)	C18, 250×4.6 mm (5 μm)	UV	189/100 g 8,333/100 g	0.04–3.73 5.5–550	Cooked meals, milk and milk products	Escrivá et al. (2002)
30	A E	76.9 mM SDS/1-BuOH (88.3:11.7, *v*/*v*) adjusted to pH 6.37 with 0.02 M phosphate buffer	C18, 100×3.9 mm (5 μm)	UV	1,710 4,520	150–1,500 2,000–8,000	Medicated syrup	Momenbeik et al. (2005)
31	A E	A: CH_3CN B: H_2O C/MeOH Gradient elution 24 min	C8. 250×4.6 mm (5 μm)	DAD	0.58 10	0.86–17.16 0.86–32.21	Polymeric diets for enteral nutrition	Kuhn et al. (2008)

BuOH butyl alcohol, *Chl* chlorophyll, *EtOH* ethyl alcohol, *H2TPP* tetraphenylporphyrin, *HFBA* heptafluorobutyric acid, *i-PrOH* isopropyl alcohol, *MeOH* methyl alcohol, *OSA* 1-octanesulfonic acid, *PnOH* amyl alcohol, *SDS* sodium lauryl sulfate, *TBME* methyl *tert*-butyl ether, *TEA* 2,2′,2″-trihydroxytriethylamine, *TFA* trifluoroacetic acid, *THF* tetrahydrofuran, *TMA* trimethylamine

Sample Preparation

Samples were taken from fruits (apricot, avocado), fruit–vegetable juices, and multivitamin diet supplements (powders and tablets). Apricots and avocados were peeled and cut into small pieces. Two grams for each product was weighed and then intermixed with 4 mL of methanol. Subsequently, samples were centrifuged for 5 min (2,000 rpm). The examined material was filtered using a nylon filter with a pore size of 0.45 μm.

One hundred milliliters of fruit–vegetable juice was taken and then 10 mL of Carrez I and 10 mL of Carrez II solutions were added for sedimentation and subsequently centrifuged for 10 min (7,000 rpm). The examined material was filtered using a nylon filter with a pore size of 0.45 μm, and then solid phase extraction (SPE) was performed. A RP18 Bakerbond column (500 mg, 3 mL) was used. The column was conditioned with methanol (1×3 mL) and water (1×3 mL). Analytes were eluted with portions of the solvents: 3 mL of 60% methanol in water, 3 mL of methanol, and

Table 2 Gradient elution parameters

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0	95	5	0.6
4	95	5	0.7
10	2	98	0.7
13	2	98	0.7
15	0	100	1.3
25	0	100	1.3

A—0.01% TFA in water; B—methanol

3 mL of chloroform. The SPE procedure was based on vitamins from multivitamin preparations extraction method (Kozhanova et al. 2002).

Two grams of diet supplements (tablet or powder) were ground, diluted in methanol, and filtered using a nylon filter with a pore size of 0.2 μm. Volumes of methanol used for dilution were matched with declared contents of analytes in the supplement. The volume of the filtered solution for every injection was 20 μL. This volume was taken three consecutive times from each analyzed sample. Matrices for standards which were used for calibration curves were prepared in the same manner as the samples.

Results and Discussion

The developed chromatographic system allows the simultaneous determination of 11 vitamins (both water- and fat-soluble). Dissociation of analytes was determined by a pK_a value for each compound and mobile phase pH value. Thus, a solution of TFA with the proper pH value was used. The pH value of the mobile phase was chosen with pK_a values of particular analytes taken into consideration to allow analyte separation. The pH value for trifluoroacetic acid with concentration of 0.01% is equal to 3. At the start of the mobile phase, gradient proportion of this acid to methanol is 95:5 (*v*/*v*).

Table 4 Retention parameters for water- and fat-soluble vitamins

Compound	k'	R	α	LOD (μg/mL)	LOQ (μg/mL)
B_1	3.35	0.93	1.01	0.96	2.90
C	4.50	2.05	1.44	0.90	2.70
B_6	8.49	16.73	1.67	0.24	0.72
B_3	8.98	4.05	1.36	0.23	0.70
B_5	12.47	8.08	1.32	0.30	0.90
B_{12}	13.19	1.91	1.07	0.41	1.22
B_9	13.39	1.40	1.05	0.85	2.55
B_2	13.99	3.49	1.03	0.83	2.51
A	19.10	41.23	1.35	1.66	4.98
D_3	22.95	51.65	1.73	5.28	15.84
E	24.04	5.26	1.81	0.61	1.85

With regard to the non-polar stationary phase, B_1 and C vitamins eluted first from the chromatographic column. The pK_a values of those compounds are 3.8 and 4.2, respectively. Applying a 0.01% solution of TFA allowed increasing the affinity of the compounds to the stationary phase in the initial part of the analysis. It allowed one to determine and elute outside of the dwell time of vitamin B_1 and C. Both flow rate of the mobile phase and temperature of the stationary phase column were also subject to examinations. Optimal analytical conditions were obtained at a temperature of 30 °C. The flow rate gradient shown in Table 2 was applied. Detection was performed with the use of a diode array detector. Thanks to this, it was possible to optimize the parameters of analytical wavelengths, which allowed decreasing interferences from the matrix. Calibration curve parameters are shown in Table 3.

The retention parameters obtained for particular analytes, capacity factor (k'), selectivity (α), and limits of detection

Table 3 Retention times and calibration curve parameters for vitamins (n=6)

Compound	Retention times (min)	Slope	Intercept	Sa	Sb	Measurement range (μg/mL)	R^2
B_1	3.36	5,632	5,407	34	1,060	11–86	0.997
C	4.51	2,259	−28,444	68	2,119	80–480	0.996
B_6	8.51	7,729	235,405	282	37,424	34–204	0.995
B_3	8.99	2,293	−691,107	89	66,487	432–936	0.994
B_5	12.48	1,572	13,685	37	1,193	204–408	0.998
B_{12}	13.20	20,253	−324,681	899	34,693	27–48	0.994
B_9	13.41	34,595	−163,720	1,082	28,866	17–34	0.996
B_2	14.00	15,516	−94,937	636	34,597	35–70	0.993
A	19.12	261	−5,492	3	306	27–108	0.999
D_3	22.96	13,556	533,484	476	51,764	70–141	0.995
E	24.05	1,549	−16,579	66	1,072	105–210	0.993

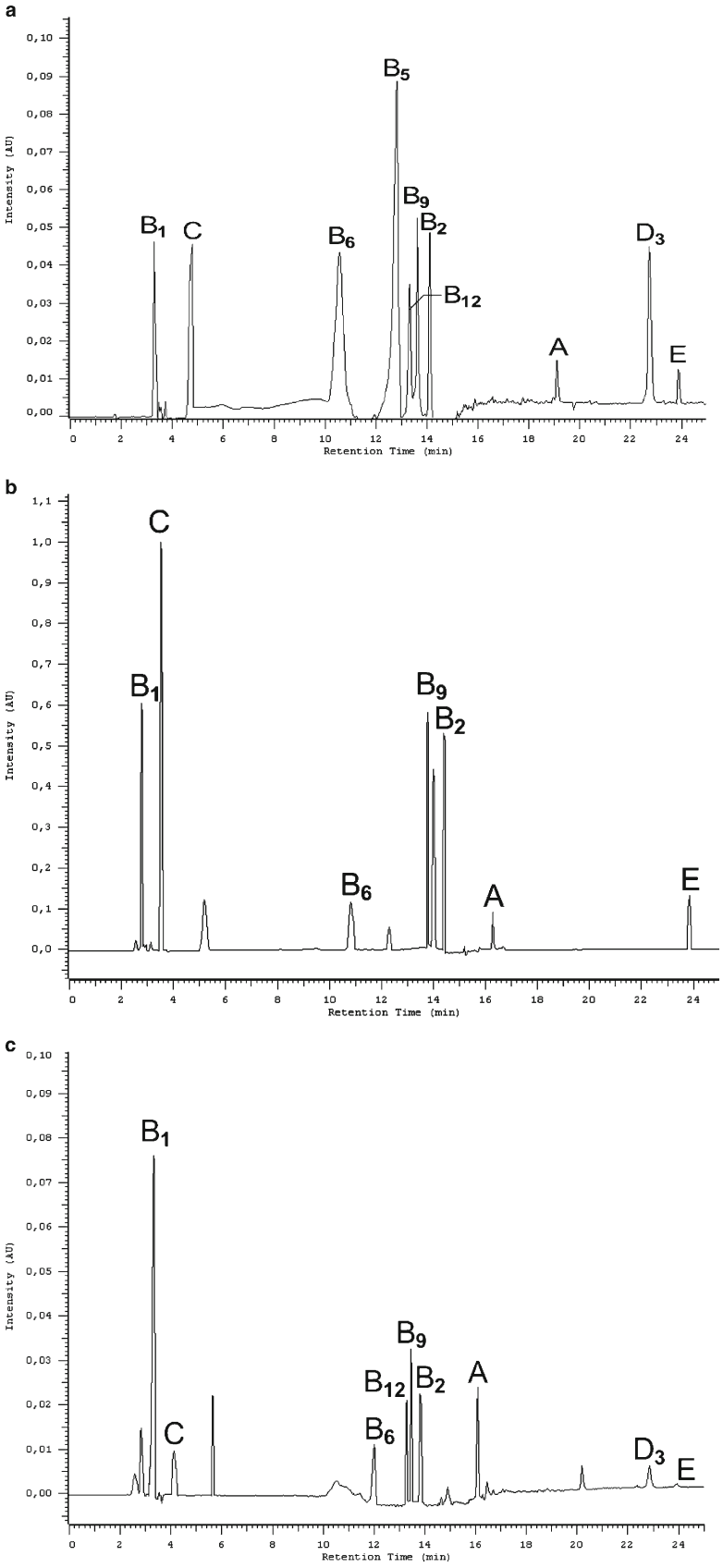

Fig. 1 **a** Chromatogram of multivitamin diet supplement solution. **b** Chromatogram of apricot extract. **c** Chromatogram of fruit–vegetable juice extract

Table 5 Precision for vitamins from fruit matrix using SLE

Compound	Input μg/100 g	Avocado				Apricot			
		Measured μg/100 g	SD μg/100 g	CV (%)	Recovery (%)	Measured μg/100 g	SD μg/100 g	CV (%)	Recovery (%)
B_1	85	79	1.44	2	93	68	1.48	2	80
C	355	305	6.15	3	86	311	6.30	3	87
B_6	61	51	2.20	1	84	54	2.05	1	88
B_3	432	382	4.30	1	88	404	4.45	1	93
B_{12}	28	–				22	0.64	2	78
B_9	20	17	0.86	2	83	18	0.80	2	90
B_2	41	37	2.28	3	91	37	2.60	3	90
A	58	48	2.70	3	83	50	2.90	3	86
D_3	127	114	1.55	1	90	102	1.21	1	80
E	110	91	3.79	3	83	88	3.81	3	80

(LOD) and quantification (LOQ) values, are shown in Table 4. Limits of detection and quantification values were determined from the signal-to-noise ratio on the assumption that the signal should be three times more intensive than the noise and the LOQ is three times higher than the LOD.

Comparing the obtained LOD and LOQ values with the literature, it can be noticed that in the works which present the application of the same detector, the LOD and LOQ values are slightly lower or comparable (Ghorbani et al. 2004; Vidović et al. 2008; Leporatia et al. 2005). However, these chromatographic systems do not allow the simultaneous determination of such number of analytes. It should also be noted that the obtained LOD and LOQ values are sufficient for the determination of the examined compounds in fruits and juices as well as in diet supplements.

The obtained selectivity factor (α) values above 1 (Table 4) mean total separation of signals for particular vitamins. The obtained LOD and LOQ values were in the range from 0.7 to 2.9 μg in 1 mL of sample for water-soluble vitamins and from 1.85 to 15.84 μg in 1 mL of sample for fat-soluble vitamins. When comparing the obtained LOD values with those from the literature for DAD, one can see that the developed chromatographic system has suitable sensitivity and can be used for the determination of all examined compounds in the chosen food and multivitamin diet supplement samples. Figure 1a shows a chromatogram example of methanol solution of a diet supplement.

Procedures for the simultaneous extraction of both fat-soluble and water-soluble vitamins from fruit and juice samples were developed. Fresh fruits were purchased in stores in Poland, multivitamin juice came from the domestic market, and diet supplements were purchased from pharmacies. For analytes, the extraction from solid matrices, solid–liquid extraction was used. Parameters of this extraction are given above ("Sample Preparation"). Examinations included apricot and avocado because they are rich in fat-soluble and water-soluble vitamins. Avocado samples contained nine vitamins: vitamin C, five vitamins of group B (B_1, B_3, B_6,

Table 6 Precision for vitamins from juice matrix using SPE

Compound	Input μg/100 g	Multivitamins juice			
		Measured μg/100 g	SD μg/100 g	CV (%)	Recovery (%)
B_1	80	75	1.42	3	94
C	355	299	6.56	4	84
B_6	61	56	3.20	4	92
B_3	432	360	5.10	3	83
B_9	18	17	0.91	2	94
B_2	41	37	2.66	2	91
A	58	50	2.05	3	86
E	110	104	3.70	2	95

B_9, B_2), and A, D_3, and E vitamins. Apricot samples additionally contained vitamin B_{12}.

Samples of fruit–vegetable juices were prepared according to the procedure specified above ("Sample Preparation"). Solid phase extraction procedure allowed extracting all eight vitamins (water-soluble C, B_1, B_3, B_6, B_9, and B_2 as well as fat-soluble A and E), which were present in the juices. Figure 1b, c shows a chromatogram example of apricot extract and a chromatogram of juice extract, respectively. The presence of analytes, in particular with real samples, was confirmed by comparing the absorption spectra in the range of 200–600 nm and adding standard solutions. Signals without labels are derived from the matrix.

Method precision was examined by analysis of recoveries from apricot, avocado, and juice matrices. The procedure for determining the value of recoveries from the solid matrix consisted of the addition of all the vitamin standards to samples of fresh fruit and then extraction with methanol. For juice samples, standard solutions were added to portions of juices, thoroughly mixed, and then representative samples were collected. Afterwards, Carrez solutions were added; the procedure was the same as for real samples. Simultaneously, whole analytical procedure for "sample without added standards" was conducted. A "sample without added standards" was a real sample of the product for which no standards solutions were added as well as no purification from the analytes was made.

The applied solid–liquid extraction (SLE) procedure achieved recoveries of water-soluble vitamins from 78% to 93% for fruit matrices. The SPE procedure achieved recoveries of water-soluble vitamins from 83% to 94% for juices matrices. For fat-soluble vitamins, recoveries were from 80% to 90% for fruits (SLE) and from 86% to 95% for multivitamin juices (SPE). Recoveries are shown in Tables 5 and 6.

Analyte contents determined in real samples of fruit, juice, and multivitamin preparation are presented in Table 7. Additionally, for the multivitamin preparation, this table contains the manufacturer's declared values. The obtained results show that the developed chromatographic procedure can be used for the simultaneous separation and determination of water- and fat-soluble vitamins in fruits, juices, and diet supplements. Problems with the determination of B_{12} and D_3 vitamins on the levels of concentration that are present in the juices and diet supplements can be eliminated by concentrating the sample before chromatographic analysis.

Conclusion

The developed chromatographic system allows the simultaneous analysis of 11 vitamins from both groups (water- and fat-soluble) in various food samples. The procedures of sample preparation are rapid and easy to perform. They allow the simultaneous extraction of all analytes and show high recovery values. Altogether, the developed chromatographic system along with the sample preparation procedures can be routinely used in food analysis. It should be emphasized that a chromatographic system together with a sample preparation procedure allows the simultaneous determination of all examined compounds in a very short time, which has never been described before.

Table 7 Contents of water- and fat-soluble vitamins in real samples of fruits, juice, and multivitamin preparation

Compound	Avocado (μg/100 g)	Apricot (μg/100 g)	Multivitamin juice (μg/100 mL)	Multivitamin preparation		SD (μg)
				Measured (μg/tab)	Declared (μg/tab)	
B_1	82.1	40.5	206.5	61.9	60	2.9
C	1,028.6	439.6	8,460.7	2,498.2	2,500	3.1
B_6	340.1	12.5	285.1	58.3	60	2.5
B_3	1,366.7	843.7	2,698.2	699.1	700	2.7
B_5	nf	nf	nf	149.5	150	2.8
B_{12}	nf	58.5	<LOQ	<LOD	0.05	3.0
B_9	71.5	16.6	28.3	–	–	1.8
B_2	186.3	62.9	256.9	68.0	70	2.6
A	187.4	227.9	246.1	31.0	30	3.1
D_3	2.8	7.4	<LOQ	<LOD	0.25	0.1
E	838.9	184.6	1,480.3	351.0	350	2.1

nf not found, *<LOD/LOQ* below LOD/LOQ

References

Berg H, Turner Ch, Dahlberg L, Mathiasson L (2000) J Biochem Biophys Methods 43:391
Breithaupt DE, Kraut S (2006) Eur Food Res Technol 222:643
Chávez-Servín JL, Castellote AI, López-Sabater MC (2006) J Chromatogr A 1122:138
Chen P, Wolf WR (2007) Anal Bioanal Chem 387:2441
Chen Z, Che B, Yao S (2006) Anal Chim Acta 569:169
Chen P, Ozcan M, Wolf WR (2007) Anal Bioanal Chem 389:343
Ching LS, Mohamed S (2001) J Agric Food Chem 49:3101
Escrivá A, Esteve MJ, Farré R, Frigola A (2002) J Chromatogr A 947:313
Franke AA, Custer LJ, Arakaki C, Murphy SP (2004) J Food Compos Anal 17:1
Frenich AG, Torres MEH, Vega AB, Vidal JLM, Bolanos PP (2005) J Agric Food Chem 53:7371
Ghorbani AR, Momenbeik F, Khorasani JH, Amini MK (2004) Anal Bioanal Chem 379:439
Gil MI, Aguayo E, Kader AA (2006) J Agric Food Chem 54:4284
Gomis DB, Fernández MP, Alvarez MDG (2000) J Chromatogr A 891:109
Heudi O, Trisconi MJ, Blake ChJ (2004) J Chromatogr A 1022:115
Heudi O, Kilinç T, Fontanna P (2005) J Chromatogr A 1070:49
Hussein A, Odumeru JA, Ayanbadejo T, Faulkner H, McNab WB, Hager H, Szijarto L (2000) Food Res Int 33:131
Kamao M, Tsugawa N, Suhara Y et al (2007) J Chromatogr B 859:192
Kienen V, Costa WF, Visentainer JV, Souza NE, Oliveira CC (2008) Talanta 75:141
Klejdus B, Petrlová J, Potěšil D et al (2004) Anal Chim Acta 520:57
Kozhanova LA, Fedorova GA, Baram GI (2002) J Anal Chem 57:40
Kozlov ÉI, Solunina IA, Lyubareva ML, Nadtochii MA (2003) Pharm Chem J 37:50
Kozlov ÉI, Solunina IA, Lyubareva ML, Nadtochii MA (2004) Pharm Chem J 38:55
Kuhn M, Nakib S, De Bandt JP, Cynober L, Loďa C (2008) J Chromatogr A 1205:186
Kurilich AC, Tsau GJ, Brown A et al (1999) J Agric Food Chem 47:1576
Lazareva EE, Brykina GD, Shpigun OA (2002) J Anal Chem 57:616
Lebiedzińska A, Szefer P (2006) Food Chem 95:116
Lebiedzińska A, Marszałł ML, Kuta J, Szefer P (2007) J Chromatogr A 1173:71
Leporatia A, Catellania D, Sumana M, Andreolib R, Maninib P, Niessenc WMA (2005) Anal Chim Acta 531:87
Lopez-Berenguer C, Martinez-Ballesta MC, Moreno DA, Carvajal M, Garcia-Viguera C (2009) J Agric Food Chem 57:572
Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner K, Baldwin EA (2006) J Agric Food Chem 54:7355
Marszałł ML, Lebiedzińska A, Czarnowski W, Szefer P (2005) J Chromatogr A 1094:91
Momenbeik F, Momeni Z, Khorasani JH (2005) J Pharm Biomed Anal 37:383
Monferrer-Pons L, Capella-Peiró ME, Gil-Agusti M, Esteve-Romero J (2003) J Chromatogr A 984:223
Moreno P, Salvadó V (2000) J Chromatogr A 870:207
Munzuroglu O, Karatas F, Geckil H (2003) Food Chem 83:205
National Academy of Sciences (1989) Washington: Food and Nutrition Board
Ndaw S, Bergaentzle M, Aoude-Werner D, Hasselmann C (2000) Food Chem 71:129
Paulo MG, Cabral Marques HM, Morais JAG, Almeida AJ (1999) J Pharm Biomed Anal 21:399
Riccio F, Mennella C, Fogliano V (2006) J Pharm Biomed Anal 41:1592
Rodas Mendoza B, Morera Pons S, Castellote Bargalló AI, López-Sabater MC (2003) J Chromatogr A 1018:197
Sikorski ZE (2007) Chemia żywności. WNT, Warszawa
Singh J, Upadhyay AK, Prasad K, Bahadur A, Rai M (2007) J Food Compos Anal 20:106
Turner Ch, Mathiasson L (2000) J Chromatogr A 874:275
Vidović S, Stojanović B, Veljković J, Pražić-Arsić L, Roglić G, Manojlović D (2008) J Chromatogr A 1202:155
Viñas P, López-Erroz C, Balsalobre N, Hernández-Córdoba M (2003) J Chromatogr A 1007:77
Wall MM (2006) J Food Compos Anal 19:434
Wang H, Cao G, Prior RL (1996) J Agric Food Chem 44:701
Xu H, Jia L (2009) J Chromatogr B 877:13